

## Epidemiological Investigation of Vaginal *Saccharomyces cerevisiae* Isolates by a Genotypic Method

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*Saccharomyces cerevisiae* is a ubiquitous, ascomycetous yeast, and vaginitis caused by this organism has been reported only very rarely. The aim of the present investigation was to assess the epidemiological relatedness of a group of vaginal and commercial *S. cerevisiae* isolates by a previously reported genetic typing method, which divided the isolates into two broad groups with numerous subtypes. Nineteen *S. cerevisiae* isolates obtained from patients suffering from vaginitis and four isolates from commercial products in the same city were analyzed. The cellular DNA from each isolate was digested with the restriction endonuclease *EcoRI*, and restriction fragment length polymorphisms were generated by horizontal gel electrophoresis. The results showed that although vaginal isolates did not cluster in any particular genetic subtype, multiple patients were infected with indistinguishable strains (there were nine distinct strains among 23 isolates). For two of three patients, all three with two episodes of *S. cerevisiae* vaginitis, different strains were isolated during the recurrence of this disease. Three other patients with indistinguishable isolates were epidemiologically related in that two were practitioners in the same clinic and the third was a patient at this clinic. We also found that one commercial strain was indistinguishable from the strain isolated from three different women at the time that they were suffering from vaginitis. The findings of the present study suggest that some *S. cerevisiae* strains may possess properties permitting persistence in the human host. Furthermore, person-to-person contact and the proliferation of the use of *S. cerevisiae* as a health-food product, in home baking, and in home brewing may be a contributing factor in human colonization and infection with this organism.

*Saccharomyces cerevisiae* is a ubiquitous, ascomycetous yeast which has been used in the preparation of food and drink for centuries and is commonly considered to be nonpathogenic. However, in the past several years this organism has been noted to be a human pathogen, particularly in immunocompromised patients (2, 9, 15, 17). Vaginitis caused by *Saccharomyces* species has been reported only rarely (10, 15, 19). The incidence of vaginitis in which this organism is the etiological agent has been estimated to be less than 1% (11).

In a review of nine patients with vaginitis caused by *S. cerevisiae*, Sobel et al. (15) found that this condition was indistinguishable from the vulvovaginitis caused by the more commonly identified fungal pathogens belonging to the genus *Candida*. Those investigators reported that *S. cerevisiae* vaginitis, although rare, tended to develop as part of a chronic syndrome as a result of local and systemic predisposing factors and that management of this condition was problematic (15). Recent investigations have assessed the pathogenicity of clinical and nonclinical isolates of this organism in an attempt to elucidate the mechanisms by which this organism causes disease (1, 3, 4, 6, 7). These investigators have reported that certain phenotypic traits are more often associated with increased virulence, in particular, an ability to grow at 42°C, an increase in the production of pseudohyphae, and possibly, a tendency to produce multiple colony phenotypes (3, 7). Hence,

there would appear to be a role for host factors as well as factors related to the organism in the pathogenesis of human disease caused by *S. cerevisiae*.

A recent study (5) used DNA typing methods to characterize 60 clinical and nonclinical isolates of *S. cerevisiae*. Those investigators devised a genetic typing method to subgroup these isolates into groups A and B on the basis of the presence or absence of a 3.0-kb band on agarose gel electrophoresis of *EcoRI*-digested DNA. Twenty-four of these isolates previously had been characterized for their degree of virulence in a murine model of systemic infection (4). Those investigators (5) reported that clinical isolates were very heterogeneous, exhibiting little clonality, that there was a statistical association of virulence with the group A DNA type, and that it was significantly more probable that vaginal isolates were of the group B DNA type.

The aim of the present investigation was to assess the epidemiological relatedness of a group of vaginal and commercial *S. cerevisiae* isolates by a previously reported genetic typing method and to test the putative association (5) of vaginal isolates with the group B genotype.

### MATERIALS AND METHODS

Isolates of *S. cerevisiae* were collected at the Genital Tract Infectious Disease Clinic at the Microbiology Institute, A. O. Ospedali Riuniti di Bergamo, Bergamo, Italy, during a 16-month period from December 1994 to March 1996. Throughout this period 4,943 patients were treated for vaginitis at this clinic.

At the time of isolation, the treating clinician completed a pro forma record for the assessment of relevant social, clinical, and laboratory information. This information included the patient's age, current symptomatology, previous history and frequency of occurrence of gynecological disease, other related medical

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TABLE 1. Clinical data and yeast intake of patients from whom the 23 *S. cerevisiae* isolates were isolated

Isolate no. <sup>a</sup>	Patient no.	Patient age (yr)	Symptoms <sup>b</sup>	No. of previous infections <sup>c</sup>	Use of yeast as follows:		
					At home <sup>d</sup>	Occupationally <sup>e</sup>	For consumption <sup>f</sup>
1	1a	29	Yes	>10	No	Yes	No
2	2	29	No	None	Yes	No	No
3	1b	29	No	>10	No	Yes	No
4	3	34	Yes	>6	Yes	No	Yes
5	4	19	Yes	None	Yes	No	Yes
6	5a	39	Yes	1	No	No	No
7	6	44	Yes	None	Yes	No	Yes
8	7	41	No	None	No	Yes	No
9	8a	25	Yes	2	Yes	No	No
10	8b	25	Yes	3	Yes	No	No
11	9	30	Yes	None	Yes	No	Yes
12	10	26	Yes	1	No	No	No
13	5b	39	Yes	1	No	No	No
14	11	36	Yes	4	No	No	No
15	12	35	Yes	None	No	No	No
16	Com <sup>g</sup>	NA <sup>h</sup>	NA	NA	NA	NA	NA
17	Com	NA	NA	NA	NA	NA	NA
18	Com	NA	NA	NA	NA	NA	NA
19	Com	NA	NA	NA	NA	NA	NA
20	13	43	Yes	None	No	No	No
21	14	49	Yes	None	No	No	No
22	15	19	Yes	1	Yes	No	No
23	16	28	Yes	None	Yes	No	Yes

<sup>a</sup> Isolates 1 and 3 (patient 1), 9 and 10 (patient 8), and 6 and 13 (patient 5) are consecutive isolates from the same patient; the strains were isolated from each patient 2, 1, and 2 months apart, respectively.

<sup>b</sup> Symptoms present at the time that the yeast was isolated.

<sup>c</sup> Previous episodes of vaginitis within the previous 2 years.

<sup>d</sup> Home use indicates the use of yeast at home in the preparation of food or beverages.

<sup>e</sup> Occupational use indicates the use of yeast in the course of the patient's employment. Patient 7 (isolate 8) was the wife of a baker.

<sup>f</sup> Consumption of large amounts of yeast for its purported health benefit.

<sup>g</sup> Com, commercial isolate.

<sup>h</sup> NA, not applicable.

history, social history, whether or not yeast products were used in the home or at work, and laboratory findings.

A total of 19 isolates of *S. cerevisiae* were recovered from the vaginas of patients suffering from vaginitis. All isolates were identified by the API 20C (bioMérieux, Marcy l'Etoile, France) system as *S. cerevisiae* with greater than 99% certainty. Each instance of isolation was of a pure culture of *S. cerevisiae* with 5 to 10 individual colonies randomly assessed and defined to the species level. For each isolation, no other organisms of known virulence in the vagina were isolated (i.e., other yeast, *Gardnerella vaginalis*, *Mobiluncus* spp., *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis*), even though the isolation of these organisms was vigorously sought.

Four additional isolates which were obtained from commercial preparations of yeasts were included in the study. These commercial isolates were obtained during the same time that the study was being conducted and were from the same region (Bergamo, Italy). All isolates were sent in a dried state to the California Institute for Medical Research, San Jose, Calif., where they were regrown for further analysis.

Standard yeast genetic techniques were used to assess spore viability and to test for species; i.e., mating-competent spores of segregants of clinical isolates were able to mate with laboratory *S. cerevisiae* strains (14). These hybrids were able to sporulate and, when dissected, yielded good spore viability and underwent meiotic recombination (5, 6, 7, 14). The ratio of segregants that fermented galactose, maltose, and raffinose and that grew on minimal medium also was assessed. The isolates were typed by morphological methods, including by the color reaction (12) and colony morphology (16). All isolates were of the rough, opaque type; two subtypes were found.

Cellular DNA was isolated by previously described methods (5, 13). Approximately 3 µg of the resultant DNA was digested with 20 U of the restriction endonuclease *EcoRI* for 6 h at 37°C. The DNA fragments were separated through a 0.7% (wt/vol) agarose gel in TAE buffer (40 mM Tris-acetate, 0.2 mM EDTA [pH 8.3]) for 20 h at 2 V/cm and were visualized by UV transillumination after ethidium bromide staining.

Digital images of the resultant DNA restriction fragment length polymorphisms (RFLPs) were captured with a charge-coupled device camera via the BioImage AQ gel documentation system (BioImage, Ann Arbor, Mich.). These images were analyzed by the BioImage AQ software, and the resultant band patterns for each of the 23 strains were matched for percent similarities by the Dice method with a 2% interband tolerance. Dendrograms were generated from

this analysis by the unweighted pair-group method with arithmetic averages (UPGMA) (8). Furthermore, digital images were captured from Polaroid photographs of the DNA RFLPs from the previous study (5) and were analyzed in the same manner to compare the similarity of the strains in the current study with those analyzed previously. From this analysis, strains with a level of similarity of >95% were considered to be the same and their *EcoRI*-digested cellular DNAs were electrophoresed side by side on the same agarose gel to confirm their identities. Those strains with <95% similarity by this methodology were considered unique.

Statistical analyses in this study used the chi-square test, and differences between groups were assumed to be significant when the probability (*P*) was less than or equal to 0.05.

## RESULTS

**Clinical correlations.** *S. cerevisiae* was isolated from 0.4% (19 of 4,943) of the samples taken from patients who were suffering from vaginitis and who presented to the Genital Tract Infectious Disease Clinic during the experimental period. As outlined in Materials and Methods, a number of individual colonies (5 to 10 colonies) were randomly assessed for each sample and were identified to the species level; in no instance were other vaginal pathogens isolated. Yeasts were cultivated from 575 (12%) of these 4,943 samples; therefore, *S. cerevisiae* was isolated 3% (19 of 575) of the time from yeast-positive samples. None of the patients from whom non-*S. cerevisiae* yeasts were isolated had *S. cerevisiae*-positive cultures.

Sixteen patients between the ages of 19 and 49 years (mean age, 27 years) were included in this study (Table 1). *S. cerevisiae* was isolated on two occasions from each of three of the patients, resulting in 19 clinical isolates that were available for evaluation (Table 1). Four nonclinical commercial isolates were obtained from the same region during the examination

period and were included for comparison (isolates 16 to 19; Table 1). In particular, the first of these isolates (isolate 16) was purchased in a general store as *Lievito del fornaio* (baker's yeast), the second (isolate 17) was purchased in a pharmacy (Sohn), and the final two isolates (isolates 18 and 19) were purchased directly from two bakeries (*Lievito verde* and *Lievito rosso*, respectively).

The majority (84%; 16 of 19) of the episodes of clinical yeast isolation occurred at times when the patients were symptomatic. For six of the 16 (38%) patients a previous episode of vaginitis had been documented during the preceding 2 years (Table 1). One-half of the patients (8 of 16) used yeast at home for the preparation or consumption of food or beverages, 31% (5 of 16) consumed yeast for its purported health benefit, one patient was a baker (she relapsed and had two episodes of *S. cerevisiae* vaginitis; patient 1, isolates 1 and 3), and a final patient (patient 7, isolate 8) was married to a baker (Table 1). Hence, in all, 69% (11 of 16) of the patients reported a known contact with a commercially available yeast. Among a control group of 26 patients who were at the same institute during the same period and who had vaginitis not associated with *Saccharomyces*, 14 had contact with a commercial yeast. Seven of these consumed yeast for medicinal purposes, and 7 used yeast at home in food preparation. Chi-square analysis comparing the study group with this control group of patients showed that there was no significant difference in the amount of contact with a commercial yeast ( $P = 0.63$ ). Furthermore, there was no significant difference between the control group and the study group in the number of patients who consumed yeast for medicinal purposes ( $P = 0.82$ ) or in the number of patients who used yeast at home in food preparation ( $P = 0.31$ ).

**Molecular typing.** The isolates were placed into genotypic subtypes according to previously published criteria (5). This method separates isolates of *S. cerevisiae* into two large groups distinguished by the presence or absence of a DNA RFLP band of approximately 3.0 kb (5). Those isolates without the 3.0-kb band were designated group A (52%; 12 of 23), and those with the 3.0-kb band were designated group B (48%; 11 of 23). The RFLPs of *EcoRI*-digested DNA of a representative sample of strains is shown in Fig. 1, which indicates the 3.0-kb band used to differentiate between groups A and B. This genotypic method of strain differentiation was used because previous results generated by this method had indicated that a disproportionate number of vaginal isolates were of the B genotypic subgroup (5).

The results of the previous study showed that 43 of 60 isolates were in group A and 17 of 60 were in group B (5). Chi-square analysis comparing the results of the previous study (5) (group A,  $n = 43$ ; group B,  $n = 17$ ) versus the present study (group A,  $n = 12$ ; group B,  $n = 11$ ) showed that the distribution of isolates into either group A or group B did not vary statistically ( $P = 0.09$ ). No association was found between vaginal isolates and group B isolates by comparing the clinical isolates of the present study (group A,  $n = 12$ ; group B,  $n = 7$ ) with the clinical isolates (group A,  $n = 35$ ; group B,  $n = 14$ ) or nonvaginal clinical isolates (group A,  $n = 33$ ; group B,  $n = 8$ ) from the previous study ( $P = 0.51$  and  $0.15$ , respectively).

**Test for spore viability and identification to the species level.** Test for sporulation of the 23 *S. cerevisiae* isolates studied showed that most strains produced spores, although the viabilities of these spores varied (Table 2). Viable spores were produced by 10 (type A<sup>v</sup>) of the 12 type A isolates, whereas 6 (type B<sup>v</sup>) of the 11 type B isolates produced viable spores. There was no significant difference in the proportion of type A<sup>v</sup> and type B<sup>v</sup> isolates in the present study ( $P = 0.14$ ) or in comparison to the proportion of the corresponding groups in

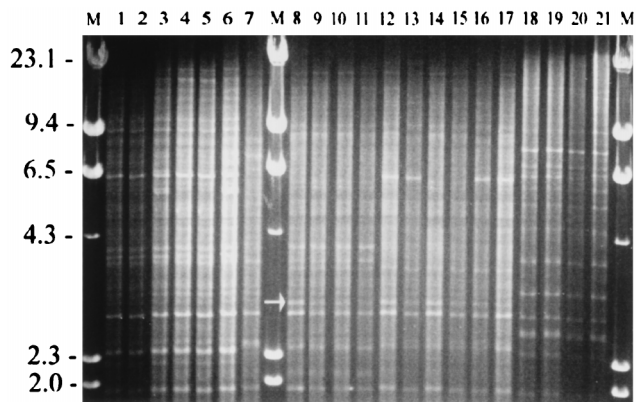


FIG. 1. Representative photograph of a UV-transilluminated, ethidium bromide-stained agarose gel. The RFLPs generated by *EcoRI* digestion of *S. cerevisiae* DNA are shown. Molecular size markers are in the lanes marked M above, and their corresponding sizes (in kilobases) are given on the left. The arrow in the middle marker lane indicates the band at approximately 3 kb that identifies the strains of the B subtype. The lanes contain DNAs from the following isolates (these numbers correspond to the isolate numbers listed in column 1, Table 1): lane 1, isolate 5; lane 2, isolate 8; lane 3, isolate 12; lane 4, isolate 20; lane 5, isolate 22; lane 6, isolate 23; lane 7, isolate 4; lane 8, isolate 21; lane 9, isolate 18; lane 10, isolate 1; lane 11, isolate 7; lane 12, isolate 16; lane 13, isolate 19; lane 14, isolate 2; lane 15, isolate 3; lane 16, isolate 9; lane 17, isolate 11; lane 18, isolate 6; lane 19, isolate 13; lane 20, isolate 14; lane 21, isolate 15.

the previous study ( $P \geq 0.2$ ) (5). No apparent correlations were found between the viabilities of the spores of the isolates and clinical findings (Tables 1 and 2). All isolates that produced viable spores were identified to the species level as *S. cerevisiae* by standard genetic tests for species determination; however, one isolate (isolate 21; Table 2) which was tetraploid or near tetraploid gave rise to diploid or near-diploid mating-competent segregants.

The genetic data presented in Table 2, plus the fermentation patterns of the isolates and segregants (data not shown), indicate genetic similarities that support the RFLP groupings. Similar to previous findings (5), all type B<sup>v</sup> isolates are *ho*, all type A<sup>v</sup> isolates are *HO* (*Ho* refers to thallism, with *HO* being homothallic and *ho* being heterothallic, and *MAT* refers to the mating genotypes in the *ho* isolates), and all isolates producing viable spores appear to be multiply heterozygous. However, five of the six type B<sup>v</sup> isolates in the present study were diploid (or near diploid) and gave rise to mating-competent haploid segregants, which is in contrast to the tetraploidy seen in the type B<sup>v</sup> isolates in the prior study (5). In general, isolates with the same DNA type had similar spore viability patterns and heterozygosities (e.g., heterozygous for *GAL*, with similar ratios of *Gal*<sup>+</sup>:*Gal*<sup>-</sup> strains). This is additional evidence that such isolates are very closely related.

## DISCUSSION

In the present study the incidence of vaginitis caused only by *S. cerevisiae* among patients suffering from vaginitis (0.4%; 19 of 4,943) was similar to that reported previously (11, 15). The patient population in the present study was from a specialty clinic to which patients with problematic conditions were referred. This is evidenced by the fact that more than a third of the patients had suffered a previous episode of vaginitis during the preceding 2 years. It has been reported that *S. cerevisiae* is more likely to be involved in chronic vaginal infections (15).

The results of the present study indicated that by these methods nine distinct strains were present among the 23 iso-

TABLE 2. DNA subtype, spore viability, and genetic test results for species of the 23 *S. cerevisiae* isolates tested

Isolate no. <sup>a</sup>	Patient no.	DNA type <sup>b</sup>	Viable spores <sup>c</sup>	No. of tetrads with the following no. of spores					Spore viability (%) <sup>d</sup>	<i>Ho</i> ( <i>Mat</i> ) <sup>e</sup>	Proof of species <sup>f</sup>
				4	3	2	1	0			
1	1a	B3	Yes	0	0	1	0	23	2	ND <sup>g</sup>	ND
2	2	B2	Yes	0	0	5	10	9	20	ho(a/a/α)	Yes
3	1b	B2	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	3	A4	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	4	A2	Yes	4	16	4	0	0	75	HO	Yes
6	5a	A5	Yes	0	11	7	4	2	53	HO	Yes
7	6	B3	No	0	0	0	0	24	0	ND	ND
8	7	A2	Yes	4	10	8	0	2	64	HO	Yes
9	8a	B4	Yes	0	0	3	4	17	10	ND	ND
10	8b	A1	Yes	0	0	5	2	18	12	ND	ND
11	9	B4	Yes	1	5	9	6	3	44	ho(a/a/α)	Yes
12	10	A3	Yes	9	10	3	2	0	77	HO	Yes
13	5b	A5	Yes	5	13	6	0	0	74	HO	Yes
14	11	A5	Yes	4	9	10	1	0	66	HO	Yes
15	12	A5	Yes	5	7	5	4	3	57	HO	Yes
16	Com <sup>h</sup>	B1	Yes	3	12	6	1	2	63	ho(a/a/α)	Yes
17	Com	B1	Yes	3	9	9	3	0	62	ho(a/a/α)	Yes
18	Com	B3	Yes	3	14	1	5	1	63	ho(a/a/α)	Yes
19	Com	B1	No	0	0	0	0	24	0	ND	ND
20	13	A2	Yes	2	14	6	1	1	65	HO	Yes
21	14	B3	Yes	5	5	8	4	2	57	ho(a/a/α)	Tetraploid
22	15	A2	Yes	2	14	8	0	0	68	HO	Yes
23	16	A3	Yes	1	12	8	2	1	60	HO	Yes

<sup>a</sup> Isolates 1 and 3 (patient 1), 9 and 10 (patient 8), and 6 and 13 (patient 5) are consecutive isolates from the same patient; the strains were isolated from each patient 2, 1, and 2 months apart, respectively.

<sup>b</sup> DNA subtype refers to whether an isolate was subtype A or B, and the digit confers identity with other strains of that subtype (11).

<sup>c</sup> Viable spores indicates whether the isolate yielded spores when tetrads were dissected.

<sup>d</sup> Spore viability indicates the percentage of viable spores from 24 dissected tetrads.

<sup>e</sup> *Ho* refers to thallism: *HO* is homothallic and *ho* is heterothallic. *Mat* refers to the *MAT* genotypes in the *ho* isolates.

<sup>f</sup> Proof of species indicates that the isolate is diploid and a member of the species *S. cerevisiae*, as determined by the production of viable progeny from a cross between a known haploid strain of *S. cerevisiae* and a mating-competent segregant of the *ho* isolates or spores of *HO* isolates.

<sup>g</sup> ND, not determined.

<sup>h</sup> Com, commercial isolate.

lates. This result is depicted in the dendrogram generated from the software analysis (Fig. 2). However, it should be noted that this dendrogram does not give any special weight to bands used for the group A and group B subgrouping. Correlation of the genetic relationships with the available clinical and social data (Table 1 and Table 2) showed several interesting findings.

First, the strains that were isolated from multiple patients with symptomatic vaginitis presumptively caused by *S. cerevisiae* were indistinguishable. In fact, 90% (17 of 19) of the isolates had a genotype that was identical to that of at least one other isolate within the present study group. Apart from a single known association (see below) there appeared to be no obvious clinical or social similarities among the patients who were infected with indistinguishable strains of *S. cerevisiae*. The exception to this finding was for the isolates from patients 5 (isolates 6 and 13), 11 (isolate 14), and 12 (isolate 15). All three of these patients had genetically indistinguishable isolates (lanes 18 to 21; Fig. 1). After the genotypic analysis was completed, further information regarding a possible relationship among these patients was sought. This uncovered the fact that two were medical practitioners in the same clinic (nongynecological), and the third was a patient at that clinic.

Second, the same strain of *S. cerevisiae* was not always isolated from a given patient. Of the three patients in the present study (patients 1, 5, and 8; Table 2) from whom an isolate was recovered at two different time points, an indistinguishable strain of *S. cerevisiae* was isolated from only one of these patients (patient 5) on both occasions; for the other two patients genetically distinct strains were isolated at the different

times. The interpretation of this latter result is interesting, because it would indicate that these patients either (i) are being serially infected with different *S. cerevisiae* strains, (ii) are being infected with a changing *S. cerevisiae* strain, or (iii) are being infected with a mixed population of strains from which only one *S. cerevisiae* strain was characterized in each instance. Without characterizing an extremely large number of individual strains from each patient, it is not possible to know if this last scenario may be true. Furthermore, it is highly unlikely that a single strain would change during the course of vaginal infection, because, with the exception of one of these vaginal isolates (Table 2), all were able to sporulate and were therefore (at least) diploid and have (at least) one copy of both *MATa* and *MATα* and so would not be able to mate. This excludes the possibility that mating of two strains with different RFLP patterns would produce a new pattern. Moreover, the expression of both *MAT* genes indicates that the *HO* gene would not be transcribed, thus eliminating the possibility of a homothallic contribution, via recombination, to alterations in the RFLP patterns. In addition, meiotic recombination would require that the strain undergo meiosis (sporulation), which many of these strains do only very inefficiently (Table 2), even under ideal laboratory conditions. Ideal sporulation conditions require a low temperature (25 to 30°C) and a highly aerobic environment, and hence, the vagina would be a very unfavorable environment for sporulation. Moreover, many of these isolates which did sporulate did not yield viable spores. Further investigation would be required to totally eliminate both the second and third possible scenarios outlined above, and the

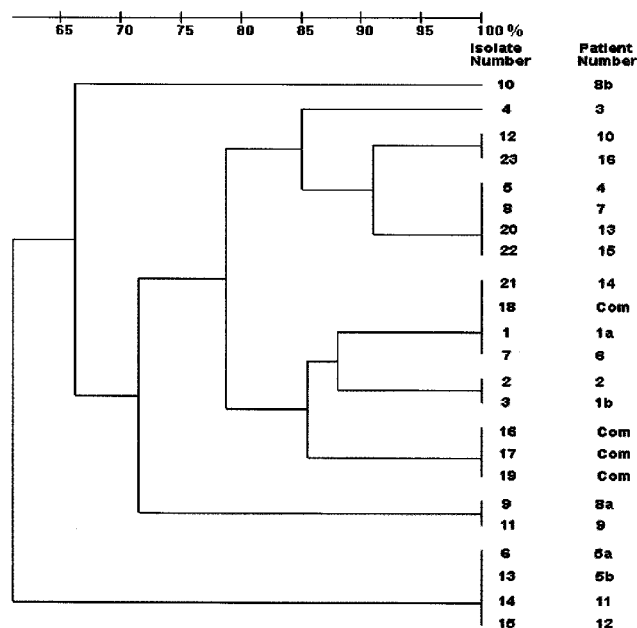


FIG. 2. Dendrogram depicting the relatedness of the 19 clinical isolates and the four commercial (Com) isolates examined in this study. Isolate and patient numbers are the same as those given in Tables 1 and 2.

investigators feel that it is most likely that these patients were infected serially with different *S. cerevisiae* strains.

Finally, one of the commercially available strains of *S. cerevisiae* (isolate 18; Table 2) was identical in all characteristics studied to the strains isolated from three different women (patients 1, 6, and 14) at the time when they were suffering from symptomatic vaginitis; these were presumed to be the same strains. The remaining three commercial strains were genetically indistinguishable from each other (isolates 16, 17, and 19; DNA type B1; Table 2). This would indicate that, at least in the geographic region studied, the commercial strains were somewhat homogeneous. The principal aim of this study was not to assess all possible commercially available yeasts at this location during the study period but only to obtain a random, representative sample. It was even more noteworthy, then, that one of these commercial yeasts was indistinguishable from that which was isolated from three patients.

Apart from those mentioned above, no association between the genotypic subgroup of the vaginal isolate and the use of a hormonal contraceptive, episodes of previous vaginitis, or sexual history (age of first coitus, homosexual versus heterosexual, type of sexual activity [oral, anal, or vaginal], and number of sexual partners) could be found. There was no correlation of genotype with morphological type or with growth on sugars. A possible exception was that 10 of 11 isolates with a droplike colonial type were of the group A genotype (versus 3 of 12 of the coral-like type). All the commercial isolates were of the coral type. These phenotypic methods would not likely be useful for any epidemiologic purposes.

The availability of photographs of the RFLPs of *EcoRI*-digested DNA generated in the previous study, which used the RFLP method for the assessment of the epidemiology of *Saccharomyces* (5), made it possible to digitize these images and compare the RFLPs of the DNAs of 60 previously analyzed *Saccharomyces* isolates with those of the isolates in the present study by using the gel analysis software. These analyses showed

that no strains were common between the two studies. In the previous study (5) 32 genotypic subgroups were found among 49 clinical isolates. A similar diversity was found among the isolates from the present study: 9 genotypic subgroups for 19 clinical isolates. Statistical analysis of the number of distinct genotypic subgroups found per number of clinical isolates studied showed that the diversity among the clinical isolates was not statistically different between the two studies ( $P = 0.18$ ).

The amount of contact that the general public has with *S. cerevisiae* is difficult to ascertain. The high incidence of home use of yeast (50%) reported here could be a cultural phenomenon, and the use of yeast for medicinal purposes (31% in the present study) may well be increasing in all Western cultures. It would appear that this amount of contact is not unusual for the study region because there was no significant difference in contact with commercial yeast between the control patients and those with vaginitis caused by *S. cerevisiae*.

A recent investigation which studied the molecular epidemiology and in vitro susceptibility patterns of clinical isolates of *S. cerevisiae* found 62 distinct DNA types among 76 clinical isolates (20). This previous investigation used pulsed-field gel electrophoresis of *NotI*-digested DNA for the molecular typing of these clinical isolates (20). Despite the genomic diversity of the isolates found by their method, those investigators identified clusters of identical isolates among different patients hospitalized concurrently in the same unit (20). These findings are similar to the results of the present study, which showed in six instances that a genetically indistinguishable strain was causing infection in multiple women. An association among the women was known for only one of these incidences. This finding is in contrast to those for other known vaginal pathogens, most notably, *Candida albicans*; it is well documented that the majority of women harbor *C. albicans* strains that are unique and that the same strain persists over prolonged periods (18). These findings raise several interesting questions.

Previous work (1, 3, 4, 6, 7) suggests that only a few non-clinical *S. cerevisiae* isolates possess properties associated with virulence. Hence, the pool of clinical isolates in Bergamo may be a subset of the *S. cerevisiae* isolates in the region, explaining the finding that the patients were infected with a small number of genetically distinct strains.

It is also not known how intimate the contact needs to be before individuals will share the same strain of *S. cerevisiae*. It would appear from the present study that sharing the same work environment and patient-to-medical doctor contact is sufficient to cause individuals to share *S. cerevisiae* strains. The first instance of transmission of *S. cerevisiae* between individuals was reported by Wilson et al. (19) in 1988. This finding was not supported by molecular epidemiological tools. The only other report of the level of intimacy required for the transmission of *S. cerevisiae* isolates between different individuals was that by Nyirjesy et al. (10). Those investigators presented evidence that the same strain of *S. cerevisiae* was isolated from the finger of the husband of one of their four patients and from the dough used in his pizza shop. This previous research would appear to be the first documentation of an industrial isolate of *S. cerevisiae* being the etiological agent of a human disease. The present study, however, may well be the first evidence that only casual contact is required for individuals to share indistinguishable strains of *S. cerevisiae* which may then cause disease.

The present study provides further evidence of a commercially available *S. cerevisiae* strain that has caused human disease; 3 of 16 cases of disease were associated with a commercial isolate. The previous report (10) used a methodology different from that used in the present study and found that

one of four cases of disease was associated with a commercial isolate. Both studies provide strong evidence that *S. cerevisiae* can be inoculated from external sources into the vagina, where it can cause a symptomatic infection.

The results presented here do not support the previously made contention (5) that certain genetic subtypes are isolated preferentially from the vagina. However, this may also be associated with geographic differences. This previous contention was based on isolates from the United States (5). None of the isolates in the present study had the same DNA RFLP type as any of the 60 isolates from the previous study (5). It may well be that significant genetic differences exist among strains of *S. cerevisiae* available on the two continents and, furthermore, that they have the ability to colonize humans and cause disease.

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